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Article

In Vitro additive effect on griseofulvin and terbinafine combinations against multidrug-resistant dermatophytes

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Griseofulvin (GF) and terbinafine (TF) are commonly used drugs to treat dermatophytosis, a fungal infection of the skin. Today there is an increase in drug resistance to these antifungals which highlight the need for alternative synergistic therapies. Minimum Inhibitory Concentration (MIC) of GF and TF were determined against fungi clinical isolates from local hospitals with values ranging 0.03–2.0 μg mL^{-1} and 0.24–4.0 μg mL^{-1}, respectively. A checkboard test was used to determine the combination of GF:TF which could induce an additive effect against the fungi isolates Multidrug-resistant isolates showed susceptibility after treatment with 16:2 μg mL^{-1} GF:TF. An MTT assay further verified that GF and TF combinations have greater additive effect against pathological and multidrug-resistant isolates than antifungals alone. Herein we disclose GF:TF combinations that could constitute as a possible new anti-dermatophyte therapy.

Keywords: Griseofulvin. Terbinafine. Dermatophyte. Multidrug-resistant fungi. Drug combination.

INTRODUCTION

Skin infections caused by dermatophytes fungus are common and widespread worldwide. The disease causing fungus result primarily from three genus *Epidermophyton*, *Microsporum,* and *Trichophyton*. These infections are typically limited to the *stratum corneum* of skin, nails, and scalp, causing tinea-like mycoses and onychomycosis characterized by the symptoms of local irritation, scaling, redness, swelling, and inflammation (Patel, Schwartz, 2011). Although rarely fatal, these fungal diseases are considered difficult to treat, since their therapy is long and frequently recur (Molina de Diego, 2011).

Fungal resistance to conventional antifungals is on the rise which further complicates the treatment (Grover, Arora, Manchanda, 2012). In addition, it is especially difficult to treat immunocompromised patients because of high toxicity associated with many antifungal agents (Vandeputte, Ferrari, Coste, 2012). Griseofulvin (GF) and terbinafine (TF) are among the most commonly used drugs for the treatment of dermatophytosis but often not effective alone (Badali *et al.*, 2015).

The current approach to treat dermatophytosis utilizes a combination of antifungals to overcome fungal resistance, especially in chronic cases (Tamura *et al*., 2014). This strategy has been successful against other diseases and is particularly attractive to the pharmaceutical industry since approved drugs gain extra lifetime.

GF and TF act on different targets in the fungal cell. GF alters DNA synthesis inhibiting mitosis by interfering with microtubules function (Kathiravan *et al.,* 2012). Whereas, TF inhibits squalene epoxidase leading to ergosterol depletion and squalene accumulation (Campoy *et al.*, 2017; Scorzoni *et al.*, 2017).

Co-administration of drugs of different mechanisms of action can often be synergist through inhibition of complementary targets inside fungal cells (Scorzoni *et al.*, 2017). This strategy has been shown to achieve a wider spectrum of antifungal activity (Mukherjee *et al.,* 2005; Campitielli *et al.*, 2017; Scorzoni *et al.*, 2017).

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Herein, we present a study of the *in vitro* effects of GF and TF combinations against multidrug-resistant fungi using fungi clinical isolates, which are simultaneously or individually resistant to GF and TF.

MATERIAL AND METHODS

Microorganisms

Clinical fungi isolates (ten strains of *T. mentagrophytes*, eleven of *T. rubrum*, eight of *M. canis*, and twelve of *M. gypseum*) were obtained from the culture collections deposited on the Laboratory of Applied Mycological Research, Universidade Federal do Rio Grande do Sul, Brazil. PCR and direct sequencing, targeting the internal transcribed spacer (ITS) region of rDNA, were used in the identification of *T. mentagrophytes* isolates employed in this study.

Antifungal solutions

Stock solutions of GF (Wallace Pharmaceuticals, Mumbai, India) and TF (terbinafine hydrochloride - Cristália, São Paulo, Brazil) were prepared by dilution with dimethyl sulfoxide (DMSO; Synth, São Paulo) at 1600 μg mL^{-1} and stored at –20 °C. For the experiments, antifungal drugs were diluted with RPMI 1640 (Roswell Park Memorial Institute; Gibco) medium supplemented with L-glutamine, without sodium bicarbonate, and buffered at pH 7.0 with MOPS (morpholinepropansulfonic acid; Sigma-Aldrich) buffer 0.165 M. After dilution, the maximum final concentration of DMSO was 2%.

Antifungal susceptibility testing

The minimum inhibitory concentration (MIC) of each antifungal drug was determined by the broth microdilution method using RPMI 1640 medium, according to CLSI protocol M38-A2 (CLSI, 2008). GF and TF resistance was defined as MIC ≥ 3 μg mL^{-1} (Galuppi *et al.,* 2010) and MIC ≥ 4 μg mL^{-1} (Mukherjee *et al.*, 2003), respectively, since no breakpoints are available in the CLSI and EUCAST for these drugs. Sterility (without drugs and fungi) and cell viability controls were used respectively and performed in triplicate.

Phenotypic Study of ATP-binding cassette (ABC) efflux pumps

MICs of GF and TF were determined to the multi-resistant isolates in the presence of verapamil (100 μM, RPMI) into the culture medium and compared with the MIC values without the addition of this efflux pump inhibitor (Pinto e Silva *et al.,* 2009). Incubation conditions were the same as those used on antifungal susceptibility tests.

Susceptibility assay of the combined antifungal drugs by the checkerboard method

Two-dimensional characterization of the GF:TF interaction was performed in quadruplicate by checkerboard technique as previously described by Lewis *et al.* (2002). Antifungal interactions were evaluated by comparing the fractional inhibitory concentration index (FICI) expressed as the sum of the fractional inhibitory concentrations (FIC), as defined by the following equation:

$$FICI = FIG_{GF} + FIG_{TF} = \frac{MIC_{GF} \text{ in combination}}{MIC_{GF} \text{ teste dalone}} + \frac{MIC_{TF} \text{ in combination}}{MIC_{TF} \text{ tested alone}}$$

where MIC_{GF} and MIC_{TF} are the MICs of GF and TF, respectively (Mukherjee *et al*, 2005). Interactions were defined as synergistic (FICI ≤ 0.5), additive (0.5< FICI < 1), indifferent (1 ≤ FICI < 4), or antagonistic (FICI ≥ 4) (Lewis *et al.,* 2002).

Cell injury test of the combined antifungal drugs

After the Checkerboard time incubation, the hypha damage caused by GF:TF association was assayed by the colorimetric test using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich) (Chiou *et al.,* 2001). The supernatant from each microplate-well was substituted with 160 μL of MTT (0.05 mg mL^{-1} – RPMI), followed by incubation at 35 °C for 24h. Next, the supernatant was replaced with 200 μl of isopropanol. Then, 100 μl from each well was transferred to another 96-well microplate for absorbance (*A*) readings at 570 and 690 nm (EnVision 2104 Multilabel Reader, PerkinElmer, USA). The cell damage (CD%) was calculated by the equation $CD\% = [1 - (A_{570nm} - A_{690\,nm}$ with drug) / ($A_{570nm} - A_{690\,nm}$ without drug)]×100.

Statistical analysis

The statistical analysis was performed by ANOVA followed by Tukey's test (Minitab 14.0 software, USA). Data expressed as mean ± SEM (Standard Error of Mean). Differences were considered statistically significant when $p<0.05$.

RESULTS

Antifungal susceptibility test

MIC and MIC_{50} values (Table I) showed that all forty-one isolates tested were more susceptible to TF than GF. GF MIC values among susceptible isolates varied from 0.25 to 2 μg mL^{-1}. Seven isolates (MCA 36, MCA 40, MGY 58, TME 16, TME 34, TRU 25, and TRU 43) were classified as resistant (MIC ≥ 3 μg mL^{-1}) to GF. TF MIC results among susceptible isolates varied from 0.03 to 2.0 μg mL^{-1}. Two isolates (TME 16 and TME 34) were classified as resistant (MIC ≥ 4 μg mL^{-1}). Finally, two isolates (TME 16 and TME 34) were identified as multi-resistant to GF and TF. Statistical analysis also demonstrated higher presence of GF-resistant than TF-resistant ($p<0.05$) dermatophytes.

Antifungal activity of the GF:TF combination

Seven isolates characterized as resistant to either GF or TF, and sixteen isolates susceptible to both antifungals were selected for this study. The FICI values calculated revealed an additive effect in about 70% of these selected strains (Table II). Multidrug-resistant isolates TME 16 and TME 34 showed to be more susceptible to GF:TF mixtures (Table II - bold) which was unexpected but an encouraging result.

Cell damage evaluation by the MTT test

Sixteen isolates, which presented additive effect in the qualitative checkerboard test (Table II) were then selected to have their ~~its~~ interaction substantiated by the quantitative MTT test The MTT test corroborates ~~to~~ the results obtained by the checkerboard assay because the GF and TF mixtures in all cases could inhibit greater amount of cell growth compared to both drugs alone. (Table III). The statistical analysis of cell damage verified that the combinations were significantly ($p<0.05$) more effective in all isolates tested.

Cell viability was evaluated in the multidrug-resistant isolate TME 16 (Figure 1 - A) and the drug sensitive isolate TME 32 (Figure 1 - B) by the MTT assay. It was found that cell damage to TME 16 was decreased by 40% after treatment with GF (32 and 16 μg mL^{-1}) and TF (2 μg mL^{-1}). In contrast, treatment with the GF:TF mixtures (16:2 and 64:2 μg mL^{-1}, respectively) resulted in much greater cell damage (>90%) indicating possible hypha death due to synergy (Figure 1 – A). Similar trend in potency improvement was noted with ~~to~~ isolate TME 32 as cell damage could be enhanced to 80% or greater after treatment with GF:TF mixtures (Figure 1 – B).

Statistical analysis of the cell damage further validate that the GF:TF combinations were significantly more effective in all isolates tested ($p<0.05$).

Phenotypic study of ABC efflux pumps

Effect of ABC efflux-pumps on potency was checked by using Verapamil (Sigma), a known efflux pump inhibitor. Drug efflux is one of the main mechanisms responsible for decrease the MIC within drug resistant organism. It was found that the MICs of GF and TF were not changed with addition of verapamil suggesting that drug efflux is not responsible for drug resistance.

DISCUSSION

According to the critical points chosen from Table I, seven out forty-one isolates assayed were classified as resistant to GF (MIC values ≥ 3 μg mL^{-1}) (Galuppi *et al.,* 2010). This finding is consistent with previously reported results (Galuppi *et al.,* 2010; Nardoni *et al.*, 2013) as was the prevalence of GF-resistant isolates compared to TF (Andes *et al.,* 2006).

Two *T. mentagrophytes* isolates (TME 16 and TME 32) showed resistance to GF and TF. This resistance to distinct antifungal drug classes is alarming, since the probability of therapeutic-treatment failure could be extremely high when these agents are given alone. The multi-drug resistance of dermatophytes to GF and TF is unusual, so further tests was performed focusing on these two multidrug-resistant isolates.

Literature has reported that one particular fungi strain (having resistance to GF, and tioconazole) which was related to the efflux pumps (Fachin, Maffei, Martinez-Rossi, 1996). Therefore, we hypothesized that TME 16 and TME 32 might be drug resistant due to the activity of efflux pumps. This was checked using verapamil to inhibit the ABC efflux pumps and MICs determined. Since the MICs were not improved upon addition of verapamil it is suggestive that the mechanism of resistance is not drug efflux. Additional studies will need to be conducted to further verify this result and uncover the mechanism of drug resistance.-

Dermatophytosis is often a chronic, recurrent disease which can require long-term treatment with antifungals. Therefore, it is important to choose the most appropriate and effective drugs in the early stages of infection. Combination therapy of antifungals with different pharmacological effects has shown to have

TABLE I - MICs to GF and TF against dermatophyte isolates

Isolates		GF (µg/mL)			TF (µg/mL)		
		MIC	Mean MIC	MIC_{50}	MIC	Mean MIC	MIC_{50}
Microsporum canis	MCA 01	1.0	8.38	0.5	0.03	0.30	0.125
	MCA 29	0.5			0.125		
	MCA 32	0.5			0.125		
	MCA 36	>32.0			1.0		
	MCA W3	0.25			0.06		
	MCA 38	0.5			0.03		
	MCA 39	0.25			0.03		
	MCA 40	>32.0			1.0		
Microsporum gypseum	MGY 42	1.0	3.91	1.0	0.03	0.24	0.06
	MGY 45	1.0			0.03		
	MGY 46	1.0			0.03		
	MGY 48	2.0			0.125		
	MGY 49	1.0			0.03		
	MGY 50	2.0			0.125		
	MGY 51	2.0			0.25		
	MGY 52	1.0			0.125		
	MGY 53	2.0			0.06		
	MGY 54	1.0			0.06		
	MGY 57	1.0			0.03		
	MGY 58	>32.0			2.0		
Trichophyton mentagrophytes	**TME 16**	**>32.0**	7.15	0.5	**4.0**	0.84	0.03
	TME 18	2.0			0.06		
	TME 31	0.5			0.03		
	TME 32	1.0			0.06		
	TME 33	0.5			0.03		
	TME 34	**>32.0**			**4.0**		
	TME 38	2.0			0.03		
	TME 40	0.5			0.03		
	TME 44	0.5			0.03		
	TME 46	0.5			0.125		
Trichophyton rubrum	TRU 20	1.0	4.45	1.0	0.06	0.12	0.03
	TRU 23	1.0			0.03		
	TRU 25	>32.0			1.0		
	TRU 40	1.0			0.06		
	TRU 42	2.0			0.03		
	TRU 43	4.0			0.03		
	TRU 46	2.0			0.03		
	TRU 48	2.0			0.03		
	TRU 49	1.0			0.03		
	TRU 50	1.0			0.06		
	TRU 52	2.0			0.03		

Isolates having MIC ≥ 4 µg/ml are classified as resistant to the drugs. **Bold**: multi-resistant isolates.

TABLE II - Fractional inhibitory concentration index (FICI) and type of interaction obtained for hyphae by testing GF:TF combinations according to the checkerboard microdilution method (qualitative methodology)

Isolates	MIC (µg/mL)		MIC Combinations (µg/mL)		FICI	Interaction
	GF	TF	GF	TF		
MCA 29	0.50	0.12	0.25	0.06	0.98	Add
MCA 32	0.50	0.12	0.25	0.06	0.98	Add
MCA 36	32.00	1.00	0.25	1.00	1.01	Ind
MCA 38	0.50	0.03	0.12	0.01	0.58	Add
MCA 39	0.25	0.03	0.06	0.01	0.57	Add
MCA 40	32.00	1.00	0.25	1.00	1.01	Ind
MGY 46	2.00	0.03	0.50	0.01	0.58	Add
MGY 48	2.00	0.12	0.12	0.06	0.54	Add
MGY 50	2.00	0.12	0.50	0.06	0.73	Add
MGY 53	2.00	0.06	0.50	0.03	0.75	Add
MGY 58	32.00	2.00	0.25	2.00	1.01	Ind
TME 16*	**64.00**	**4.00**	**16.00**	**2.00**	**0.75**	**Add**
TME 18	2.00	0.06	1.00	0.06	0.67	Add
TME 32	1.00	0.06	0.06	0.03	0.56	Add
TME 33	0.50	0.03	0.03	0.03	1.06	Ind
TME 34*	**64.00**	**4.00**	**16.00**	**2.00**	**0.75**	**Add**
TRU 20	1.00	0.06	0.125	0.03	0.63	Add
TRU 25	32.00	1.00	0.25	1.00	1.01	Ind
TRU 42	2.00	0.03	1.00	0.007	0.73	Add
TRU 46	2.00	0.06	0.50	0.03	0.75	Add
TRU 48	2.00	0.03	0.25	0.03	1.01	Ind
TRU 50	1.00	0.06	0.50	0.03	1.00	Ind
TRU 52	2.00	0.06	0.25	0.03	0.63	Add

Synergistic (Syn); additive (Add); indifferent (Ind). *multi-resistant isolates.

TABLE III - Cell damage after treatment with GF, TF, and GF:TF combinations (quantitative methodology)

Strains		C (µg/mL) GF, TF CDA (%) GF, TF CDC (%) GF:TF Combinations					
		Comb 1		Comb 2		Comb 3	
MCA 29	C	0.25	0.06				
	CDA	27.9% ^{C}	42.7 ^{B}				
	CDC	**80.9** ^{A}					
MCA 32	C	0.25	0.06				
	CDA	62.6% ^{B}	51.2 ^{C}				
	CDC	87.3 ^{A}					
MCA 38	C	0.25	0.01	0.125	0.01	0.25	0.007
	CDA	55.3% ^{B}	63.1 ^{B}	37.2% ^{C}	63.1 ^{B}	55.3% ^{B}	39.3 ^{C}
	CDC	89.6 ^{A}		88.7 ^{A}		88.0 ^{A}	

TABLE III - Cell damage after treatment with GF, TF, and GF:TF combinations (quantitative methodology) (cont.)

Strains		C (μg/mL) GF, TF CDA (%) GF, TF CDC (%) GF:TF Combinations					
		Comb 1		Comb 2		Comb 3	
	C	0.25	0.01	0.125	0.01		
MCA39	CDA	36.2% ^{B}	37.1 ^{B}	35.2% ^{B}	37.1 ^{B}		
	CDC	**82.6** ^{A}		**80.5** ^{A}			
	C	1.0	0.125	0.125	0.5		
MGY 46	CDA	20.7% ^{D}	36.3 ^{C}	36.3 ^{C}	11.4% ^{E}		
	CDC	**87.7** ^{A}		**80.2** ^{A}			
	C	1.0	0.06	0.25	0.06	0.125	0.06
MGY 48	CDA	64.9% ^{B}	70.9 ^{B}	21.1% ^{D}	70.9 ^{B}	29.9% ^{CD}	70.9 ^{B}
	CDC	91.7 ^{A}		90.3 ^{A}		88.9 ^{A}	
	C	0.5	0.06	0.06	0.25		
MGY 50	CDA	57.9% ^{B}	53.2 ^{B}	53.2 ^{B}	34.8% ^{C}		
	CDC	90.1 ^{A}		82.5 ^{A}			
	C	1.0	0.03	0.5	0.03		
MGY 53	CDA	52.8% ^{C}	27.0 ^{D}	3.9% ^{E}	27.0 ^{D}		
	CDC	**92.7** ^{A}		**80.9** ^{B}			
	C	32.0	2.0	16.0	2.0		
TME 16	CDA	35.1% ^{A}	30.9 ^{B}	6.8% ^{A}	30.9 ^{B}		
	CDC	**94.2** ^{C}		**90.3** ^{C}			
	C	1.0	0.06	1.0	0.03		
TME 18	CDA	67.6% ^{C}	63.5 ^{C}	67.6% ^{C}	41.1 ^{D}		
	CDC	90.1 ^{A}		81.7 ^{B}			
	C	0.25	0.03	0.125	0.03	0.06	0.03
TME 32	CDA	45.4% ^{B}	30.5 ^{C}	37.9% ^{BC}	30.5 ^{C}	31.5% ^{C}	30.5 ^{C}
	CDC	**83.8** ^{A}		**81.6** ^{A}		**80.6** ^{A}	
	C	32.0	2.0	16.0	2.0		
TME 34	CDA	48.7% ^{B}	35.5 ^{B}	47.7% ^{B}	35.5 ^{B}		
	CDC	82.9 ^{A}		81.4 ^{A}			
	C	0.5	0.03	0.25	0.03	0.125	0.03
TRU 20	CDA	77.7% ^{BC}	67.5 ^{C}	23.5% ^{D}	67.5 ^{C}	3.9% ^{E}	67.5 ^{C}
	CDC	90.5 ^{A}		**93.7** ^{A}		**83.1** ^{AB}	
	C	1.0	0.015	1.0	0.007		
TRU 42	CDA	55.1% ^{C}	52.5 ^{C}	55.1% ^{C}	21.4 ^{D}		
	CDC	87.1 ^{A}		**81.4** ^{B}			
	C	1.0	0.03	0.5	0.03		
TRU 46	CDA	55.4% ^{B}	41.5 ^{B}	37.6% ^{B}	41.5 ^{B}		
	CDC	81.2 ^{A}		**80.6** ^{A}			
	C	1.0	0.03	0.5	0.03	0.25	0.03
TRU 52	CDA	79.5% ^{B}	79.1 ^{B}	33.4% ^{DE}	79.1 ^{B}	26.6% ^{E}	79.1 ^{B}
	CDC	90.7 ^{A}		91.4 ^{A}		90.8 ^{A}	

Microsporum canis (MCA); *Microsporum gypseum* (MGY)*; Trichophyton mentagrophytes* (TME)*; Trichophyton rubrum* (TRU). C: concentration to each antifungal.CDA: percentage of cell damage to GF or TF. CDC: percentage of cell damage to GF:TF combinations (C_{comb}=[GF]+[TF]). Comb 1-3: concentrations of GF:TF combinations to result in cellular damage over 80%. Bold: indication of synergist effect. Two superscript letters represents significance $p < 0.05$ between treatments. One letter relates to no significant difference.

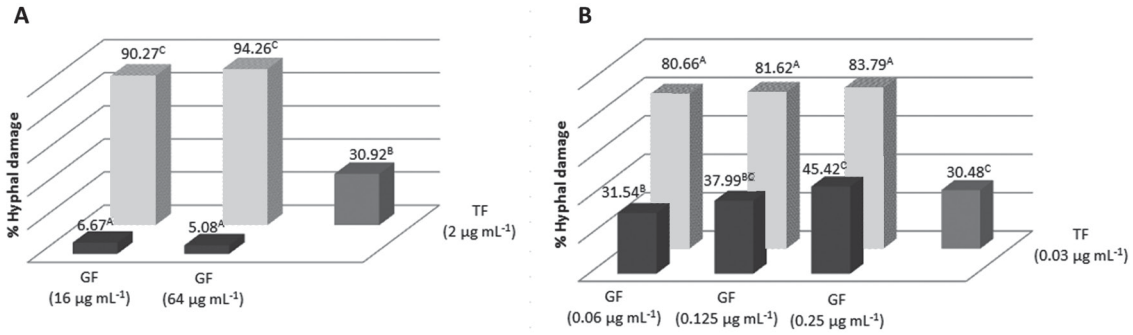

FIGURE 1 - Hyphal damage expressed in percentage of the griseofulvin (GF) (■) and terbinafine (TF) (■) alone and in combination (■). **A)** Hyphal damage of the TME 16 drug resistant isolate to the combination of TF and GF antifungals at concentrations below MIC; **B)** Hyphal damage of TME 32 drug sensitive isolate to combination of TF and GF antifungals at concentrations below MIC. Different letters above the hyphal damage represent a statistically significant difference ($p < 0.05$).

merit for treatment of dermatophytosis (Scorzoni *et al.,* 2017).

TF combined with amorolfine was checked in a randomized study of severe dermatophyte onychomycosis. Patients outcome were good and this combination treatment demonstrated an improvement cost per cure ratio (Baran *et al.,* 2000). Onychomycosis caused by dermatophytes were also successfully treated by combination of oral terbinafine with ciclopyroxolamine, imidazole and other such topical antifungals. (Romano *et al.,* 2005). *Fusarium oxysporum* infections respond positively to TF and topical imidazole treatment and this combination led to clinical and mycological healing, which is often refractory to antimycotics (Romano *et al.,* 2005). Patient recovery to dermatophytosis was also achieved when GF plus cyclopyroxolamine lotion and GF with topical imidazole were chosen and used in combination therapy (Romano *et al.,* 2005). Several other studies of combination therapy were reported, however most of these studies were lacking quantitative data regarding drug combinations *in vitro* (Spader *et al.,* 2013; Semis *et al.,* 2015).

The qualitative checkerboard assay enabled us to identify combinations of GF and TF which resulted in additive drug effects against sixteen pathogenic fungi (Table II). Subsequent evaluation by the quantitative MTT test using the same sixteen isolates identified by the checkerboard assay was performed and resulted in the determination of GF:TF concentrations that caused over 80% of cellular damage (Figure 1).

Of great importance was discovery of GF:TF drug combination that could cause 90% and 80% cellular damage to multidrug-resistant isolates TME 16 and TME 32, respectively.

It is possible that additive effect was achieved based upon the mechanism of action of GF (nucleic acid inhibitor) and TF (ergosterol synthesis inhibitor). Alteration of the membrane integrity can be linked to squalene epoxidase inhibition by TF, which would promote cellular internalization of GF; while intracellular accumulation would decreases DNA synthesis (Favre, Ryder, 1997; Polak, 1993).

While this is only one biochemical hypothesis, it is also possible that intermolecular bonds in between GF and TB molecules could be responsible for additive effect observed. Our group will be pursuing additional studies to identify these possible interactions and the results will be reported in the due course.

As reported previously we found that GF has low solubility in water (Kahsav *et al.*, 2013). In fact, it was noted that GF partially precipitates in aqueous solution at 35 °C. Consequently, it was necessary to mediate drug precipitation during incubation to avoid misleading results. This was first attempted by using MOPS as a buffer to impart enhanced compound solubility.

However, it is not possible to make the desired stock solutions of GF in concentrations higher than 10 µg mL^{-1} in MOPS buffer at 35 °C described at the CLSI for MIC determinations (CLSI, 2008). As an alternative, we found that we could make stock solution 1.6 mg mL^{-1} of GF in DMSO and dilution were made with RPMI 1640 media thereby allowing for proper MICs determinations.

In conclusion, herein we report the first investigation of GF and TF combination drug assessments against a panel of fungi clinical isolates. While, these two drugs have been evaluated with other antifungals (Baran *et al.,* 2000; Romano *et al.,* 2005) our findings are new and noteworthy particularly when pathological dermatophytes have become drug resistant to many antifungal drugs (including GF and TB alone). Through a checkerboard assay, combining GF and TF results in additive effect and has impressive efficacy to several clinical dermatophytes

including two hard to kill multidrug-resistant fungi isolates. This enhanced activity might be due to having selected drugs of different mechanisms of actions: inhibition of mitosis (GF) and ergosterol depletion (TF), as it is believed that TF might be altering the membrane by blocking the squalene epoxidase which ultimately facilitates cellular internalization of GF allowing for re-sensitizing via DNA damage of the multidrug-resistant isolates.

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